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	TYPE OF SEARCH:	VENDOR/COST (where applic.)
Searcher:_____	NA Sequences:_____	STN:_____
Phone:_____	AA Sequences:_____	DIALOG:_____
Location:_____	Structures:_____	Questel/Orbit:_____
Date Picked Up:_____	Bibliographic:_____	DRLink:_____
Date Completed:_____	Litigation:_____	Lexis/Nexis:_____
Searcher Prep/Review:_____	Full text:_____	Sequence Sys.:_____
Clerical:_____	Patent Family:_____	WWW/Internet:_____
Online time:_____	Other:_____	Other (specify):_____

	L #	Hits	Search Text	DBs	Time Stamp
1	L1	36	sphingosine adj kinase$	USPAT; US-PGPUB	2003/05/23 14:17
2	L2	17	1 same (gene$1 or sequence$1)	USPAT; US-PGPUB	2003/05/23 14:18

PGPUB-DOCUMENT-NUMBER: 20030096022

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030096022 A1

TITLE: Compositions and methods for the treatment and prevention of cardiovascular diseases and disorders, and for identifying agents therapeutic therefor

PUBLICATION-DATE: May 22, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Sabbadini, Roger A.	Lakeside	CA	US	

APPL-NO: 10/ 029372

DATE FILED: December 21, 2001

RELATED-US-APPL-DATA:

non-provisional-of-provisional 60257926 20001222 US

US-CL-CURRENT: 424/725

ABSTRACT:

Methods and compositions are disclosed that are useful for the prevention and/or treatment of cardiovascular and cardiac diseases and disorders, or damage resulting from surgical or medical procedures that may cause ischemic or ischemic/reperfusion damage in humans; and cardiovascular trauma. The beneficial effects of the compositions and methods are achieved through the use of pharmaceutical compositions that include agents that interfere with the production and/or biological activities of sphingolipids and their metabolites, particularly sphingosine (SPH) and sphingosine-1-phosphate (S-1-P). Also disclosed are methods for identifying and isolating therapeutic agents.

RELATED APPLICATIONS

[0001] This application claims priority to U.S. patent application Serial No. 60/257,926 entitled "Compositions and Methods for the Treatment and Prevention of Cardiac and Myocardial Disorders" by Sabbadini, Roger A., filed Dec. 22, 2000.

[0002] This application is related to U.S. patent application Ser. No. _____ (attorney docket No. 078853-0304), Ser. No. _____ (attorney docket No. 078853-0305), and Ser. No. _____ (attorney docket No. 078853-0306), each entitled "Compositions and Methods for the Treatment and Prevention of Cardiovascular Diseases and Disorders, and for Identifying Agents Therapeutic Therefor" by Sabbadini, Roger A., and filed Dec. 21, 2001.

[0003] All of the preceding applications are hereby incorporated in their entirety by reference thereto.

---------- KWIC ---------

Detail Description Paragraph - DETX (285):

[0324] **Sphingosine Kinase** (SPH kinase) catalyzes the conversion of SPH to S-1-P (Rxn. #3 in FIG. 2; see also FIG. 1). A genetic **sequence** encoding human SPH-kinase has been described (Melendez et al., **Gene** 251:19-26, 2000). Three human homologs of SPH kinase (SKA, SKB and SKC) have been described (published PCT patent application WO 00/52173). Murine SPH kinase has also been described (Kohama et al., J. Biol. Chem. 273:23722-23728, 1998; and published (PCT patent application WO 99/61581). Published PCT patent application WO 99/61581 to Spiegel is stated to describe nucleic acids encoding a **sphingosine kinase**. Published PCT patent application WO 00/52173 to Munroe et al. is stated to describe nucleic acids encoding homologues of **sphingosine kinase**. Other SPH Kinases are described by Pitson et al., "Human **sphingosine kinase**: purification, molecular cloning and characterization of the native and recombinant enzymes", Biochem J. 350:429-441, 2000; and published PCT application WO 00/70028 to Pitson et al.; and Liu et al., "Molecular Cloning and Functional Characterization of a Novel Mammalian **Sphingosine Kinase** Type 2 Isoform", The Journal of Biological Chemistry, 275:19513-19520, 2000; Vadas et al., "**Sphingosine Kinase** and Uses Thereof", PCT/AU01/00539, published as WO 01/85953 on Nov. 15, 2001; Rastelli, "Novel **Sphingosine Kinases**", PCT/US01/04789, published as WO 01/60990 on Aug. 23, 2001; Allen et al., "Human **Sphingosine Kinase Gene**", PCT/EP00/09498, published as WO 01/31029 on May 3, 2001.

PGPUB-DOCUMENT-NUMBER: 20030082511

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030082511 A1

TITLE: Identification of modulatory molecules using inducible promoters

PUBLICATION-DATE: May 1, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Brown, Steven J.	San Diego	CA	US	
Dunnington, Damien J.	San Diego	CA	US	
Clark, Imran	San Diego	CA	US	

APPL-NO: 09/ 965201

DATE FILED: September 25, 2001

US-CL-CURRENT: 435/4, 435/6

ABSTRACT:

Methods for identifying an ion channel modulator, a target membrane receptor modulator molecule, and other modulatory molecules are disclosed, as well as cells and vectors for use in those methods. A polynucleotide encoding target is provided in a cell under control of an inducible promoter, and candidate modulatory molecules are contacted with the cell after induction of the promoter to ascertain whether a change in a measurable physiological parameter occurs as a result of the candidate modulatory molecule.

---------- KWIC ---------

Detail Description Table CWU - DETL (23):

secretory leukocyte protease inhibitor (antileukoproteinase); HUSI-I SMA@ "SMA; spinal muscular atrophy (Werdnig-Hoffmann disease, Kugelberg- Welander disease)" SMARCA3 "SNF2L3; SNF2 (sucrose nonfermenting, yeast, homolog)-like 3; SWI/SNF related, matrix associated, actin dependent regulator of chromatin, subfamily a, member 3; HLTF; HIP116; helicase-like transcription factor" SMARCB1 "SNFSL1; SWI/SNF related, matrix associated, actin dependent regulator of chromatin, subfamily b, member 1; INI1; SNF5 (sucrose nonfermenting, yeast, homolog)-like 1 (integrase interactor 1); Snrl; BAF47; hSNFS; Sfh1p" SMPD1 "sphingomyelin phosphodiesterase 1, acid lysosomal (acid sphingomyelinase); Hs.77813; Niemann-Pick disease" SMPD2 "sphingomyelin phosphodiesterase 2, neutral membrane (neutral sphingomyelinase); nSMase" SMS spermine synthase; SpS SNCA "PARK 1; synuclein, alpha (non A4 component of amyloid precursor); Parkinson disease, familial 1; Hs.76930; NACP; PD1" SNK

serum-inducible kinase SOAT1 SOAT; Hs.172; STAT; ACAT; sterol O-acyltransferase (acyl-Coenzyme A: cholesterol acyltransferase); ACAT-1 SOAT2 sterol O-acyltransferase 2; ACAT2; ARGP2; sterol O-acyltransferase 2 SOD1 "superoxide dismutase 1, soluble (amyotrophic lateral sclerosis 1 (adult)); Hs.75428; ALS; ALS1" SOD2 "Hs.73830; superoxide dismutase 2, mitochondrial" SOD3 "Hs.2420; superoxide dismutase 3, extracellular" SORD Hs.878; sorbitol dehydrogenase SP-22 thioreductase-dependent peroxide reductase SP-22 SPAM1 "sperm adhesion molecule 1 (PH-20 hyaluronidase, zona pellucida binding); PH-20; HYAL3" SPC18 signal peptidase complex (18 kD) SPHAR s-phase response **gene** SPHK1 **sphingosine kinase** 1 SPINK1 "Hs.46262; serine protease inhibitor, Kazal type 1" SPINK2 "HUSI-II; serine protease inhibitor, Kazal type 2 (acrosin-trypsin inhibitor)" SPINT1 "serine protease inhibitor, Kunitz type 1" SPINT2 "KOP; HAI-2; serine protease inhibitor, Kunitz type, 2" SPINT3 "HKIB9; serine protease inhibitor, Kunitz type, 3" SPR "sepiapterin reductase (7,8-dihydrobiopterin:NADP+ oxidoreductase)" SPS2 selenophosphate synthetase 2 SPTI LCB1; serine palmitoyltransferase subunit I SPUVE "serine protease, umbilical endothelium" SQLE squalene epoxidase SRD5A1 "steroid-5-alpha-reductase, alpha polypeptide 1 (3-oxo-5 alpha-steroid delta 4-dehydrogenase alpha 1); Hs.552" SRD5A2 "steroid-5-alpha-reductase, alpha polypeptide 2 (3-oxo-5 alpha-steroid delta 4-dehydrogenase alpha 2); Hs.1989" SRD5AP1 "steroid-5-alpha-reductase, alpha polypeptide pseudogene 1 (3-oxo-5 alpha-steroid delta 4-dehydrogenase alpha pseudogene)" SRD5BP1 "steroid-5-beta-reductase, beta polypeptide pseudogene 1" SRM Hs.76244; SRML1; spermidine synthase SRML2 spermidine synthase-like 2 SRMS SRM; src-related kinase lacking C-terminal regulatory tyrosine and N- terminal myristylation sites SRPK1 SFRS protein kinase 1; SFRSK1 SRPK2 SFRS protein kinase 2; SFRSK2 ST3GALVI "alpha2,3-sialyltransferase" STAT3 signal transducer and activator of transcription 3 (acute-phase response factor); Hs.1618; APRF STE "sulfotransferase, estrogen-preferring; EST" STGD2 Stargardt disease 2 (autosomal dominant) STGD3 Stargardt disease 3 (autosomal dominant) STGD4 Stargardt disease 4 (autosomal dominant) STHM sialyltransferase STK10 serine/threonine kinase 10; LOK STK11 serine/threonine kinase 11 (Peutz-Jeghers syndrome); PJS; LKB1 STK12 AIK2; ARK2; ATM-1; serine/threonine kinase 12 STK13 serine/threonine kinase 13 (aurora/IPL1-like) STK14A serine/threonine kinase 14 alpha; p70S6k STR15 serine/threonine kinase 15; BTAK; serine/threonine kinase 15 STK16 MPSK; PKL12; serine/threonine kinase 16 STK17A DRAK1; serine/threonine kinase 17a (apoptosis-inducing) STK17B DRAK2; serine/threonine kinase 17b (apoptosis-inducing) STK18 serine/threonine kinase 18 STK19 serine/threonine kinase 19; D6S974E; D6S60; D6S60E; RP1; G11 STK2 Hs.1087; serine/threonine kinase 2 STK3 "serine/threonine kinase 3 (Ste20, yeast homolog); MST2; KRS1" STK4 "serine/threonine kinase 4 (Ste20, yeast homolog); MST1; KRS2" STK6 serine/threonine kinase 6; aurora IPL1-like kinase; BTAK; AIK STK6P serine/threonine kinase 6 pseudogene; STK6P1 STK9 serine/threonine kinase 9 STS "ARSC1; ARSC; Hs.79876; arylsulfatase C, isozyme S; steroid sulfatase (microsomal)" STSP steroid sulfatase (microsomal) pseudogene SUCLA2 "succinate-CoA ligase, ADP-forming, beta subunit" SUCLG1 "SUCLA1; succinate-CoA ligase, GDP-forming, alpha subunit" SUCLG2 "succinate-CoA ligase, GDP-forming, beta subunit" SULT sulfotransferase SULT1A1 "STP1; sulfotransferase family 1A, phenol-preferring, member 1; STP; P-PST; sulfotransferase, phenol-preferring 1" SULT1A2 "STP2; sulfotransferase family 1A, phenol-preferring, member 2; sulfotransferase, phenol-preferring 2; HAST4" SULT1A3 "STM; sulfotransferase family 1A, phenol-preferring, member 3;

TL-PST; sulfotransferase, monoamine-preferring" SULT1C1 sulfotransferase 1C1 SULT1C2 SULT1C sulfotransferase SULT2A1 "STD; sulfotransferase family 2A, dehydroepiandrosterone (DHEA)- preferring, member 1; Hs.81884; DHEA-ST; sulfotransferase, dehydroepiandrosterone (DHEA)-preferring" SULT2B1 "sulfotransferase family 2B, member 1; HSST2" SUOX sulfite oxidase SURB7 "SRB7; SRB7 (suppressor of RNA polymerase B, yeast) homolog" SYK Hs.74101; spleen tyrosine kinase SYNGAP "synaptic Ras GTPase activating protein, 135-kD, rat, homolog of" SYNJ1 synaptojanin 1; inositol 5'-phosphatase (synaptojanin 1); INPP5G SYNJ2 synaptojanin 2; inositol phosphate 5'-phosphatase 2 (synaptojanin 2); INPP5H TACTILE "T cell activation, increased late expression" TADA3L "ADA3; transcriptional adaptor 2 (ADA2, yeast homolog)-3 like (PCAF histone acetylase complex)" TAF1A "SL1; TAFI48; TATA box binding protein (TBP)-associated factor, RNA polymerase I, A, 48 kD" TAF1B "SL1; TAFI63; TATA box binding protein (TBP)-associated factor, RNA polymerase I, B, 63 kD" TAF1C "SL1; TAFI95; TAFI110; TATA box binding protein (TBP)-associated factor, RNA polymerase I, C, 110 kD" TAF2A "CCG1; BA2R; TATA box binding protein (TBP)-associated factor, RNA polymerase II, A, 250 kD; CCGS; NSCL2; TAFII250; BALB/c 3T3 ts2 temperature sensitivity complementing; cell cycle, G1 phase defect, (transcription factor TFIID p250 polypeptide)" TAF2B "TATA box binding protein (TBP)-associated factor, RNA polymerase II, B, 150 kD; TAFII150" TAF2C1 "TAF2C; TATA box binding protein (TBP)-associated factor, RNA polymerase II, C1, 130 kD; TAFII130; TAFII135" TAF2C2 "TATA box binding protein (TBP)-associated factor, RNA polymerase II, C2, 105 kD; TAFII105" TAF2D "TATA box binding protein (TBP)-associated factor, RNA polymerase II, D, 100 kD; TAFII100" TAF2E "TATA box binding protein (TBP)-associated factor, RNA polymerase II, B, 70/85 kD; TAFII70; TAFII85" TAF2F "TAFII55; TATA box binding protein (TBP)-associated factor, RNA polymerase II, F, 55 kD" TAF2G "TATA box binding protein (TBP)-associated factor, RNA polymerase II, G, 32 kD; TAFII31; TAFII32" TAF2H "TATA box binding protein (TBP)-associated factor, RNA polymerase II, H, 30 kD; TAF2A; TAFII30" TAF2I "TATA box binding protein (TBP)-associated factor, RNA polymerase II, I, 28 kD; TAFII28" TAF2J "TATA box binding protein (TBP)-associated factor, RNA polymerase II, J, 20 kD; TAFII20" TAF2K "TATA box binding protein (TBP)-associated factor, RNA polymerase II, K, 18 kD; TAFII18" TAF3A "TAFIII134 TATA box binding protein (TBP)-associated factor, RNA polymerase III, A, 134 kD" TAF3B "TAFIII120; TATA box binding protein (TBP)-associated factor, RNA polymerase III, B, 120 kD" TAF3D "TAFIII80; TATA box binding protein (TBP)-associated factor, RNA polymerase III, D, 80 kD" TALDO1 transaldolase 1 TALDOP1 TALDO; transaldolase pseudogene 1; Hs.77290; TAL-H TAO1 KIAA0881; thousand and one amino acid protein kinase TARS

PGPUB-DOCUMENT-NUMBER: 20030054452

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030054452 A1

TITLE: Isolated human EDG-4 receptor

PUBLICATION-DATE: March 20, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Munroe, Donald G.	Waterdown		CA	
Kamboj, Rajender	Mississauga		CA	
Peters, Diana	Toronto		CA	
Kooshesh, Fatemeh	Etobicoke		CA	
Vyas, Tejal B.	Mississauga		CA	
Gupta, Ashwani K.	Mississauga		CA	

APPL-NO: 10/ 084507

DATE FILED: February 28, 2002

RELATED-US-APPL-DATA:

child 10084507 A1 20020228

parent continuation-of 09222995 19981230 US ABANDONED

non-provisional-of-provisional 60070185 19971230 US

non-provisional-of-provisional 60080610 19980403 US

non-provisional-of-provisional 60109885 19981125 US

US-CL-CURRENT: 435/69.1, 435/320.1 , 435/325 , 435/7.21 , 530/350 , 536/23.5

ABSTRACT:

A novel, isolated EDG receptor that upon activation results in increased induction of IL-8 or NF-.kappa.B. Preferably, the EDG receptor is a human EDG-4 receptor, which has an amino acid sequence shown in FIGS. 16A and 16B, or a variant of these sequences having at least 91% sequence identity.

---------- KWIC ---------

Detail Description Paragraph - DETX (333):

[0358] Like p75.sup.NGFR, several other death receptors have been shown to induce apoptosis and/or NF-.kappa.B activation, depending on the cell type and

costimulus applied. The involvement of sphingomyelinase, ceramide/sphingosine and **sphingosine kinase** in the signaling cascade has also been shown repeatedly with TNFR, Fas/CD95 and other family members. Another parallel with the NGF system is the observation that some cell types that express a given death receptor survive their ligands while other do not. Again, protein kinase C is implicated in survival pathways. There is even direct evidence that S1P plays a similar role in survival for Fas/CD95 and inflammatory **gene** expression for TNFR. Therefore, one can predict a widespread role for inflammatory lysosphingolipid/edg receptors in modulating the apoptotic/inflammatory potential of death receptor ligands. If true, these GPCRs may play a fundamental role in cell survival, differentiation, and inflammation. Therefore, methods for isolating such receptors, and for identifying ligands at modulate these activities constitute aspects of the invention described herein.

PGPUB-DOCUMENT-NUMBER: 20030027304

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030027304 A1

TITLE: Compositions and methods for the treatment and prevention of cardiovascular diseases and disorders, and for identifying agents therapeutic therefor

PUBLICATION-DATE: February 6, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Sabbadini, Roger A.	Lakeside	CA	US	

APPL-NO: 10/ 029401

DATE FILED: December 21, 2001

RELATED-US-APPL-DATA:

non-provisional-of-provisional 60257926 20001222 US

US-CL-CURRENT: 435/184, 435/320.1 , 435/325 , 435/69.2 , 536/23.2

ABSTRACT:

Methods and compositions are disclosed that are useful for the prevention and/or treatment of cardiovascular and cardiac diseases and disorders, or damage resulting from surgical or medical procedures that may cause ischemic or ischemic/reperfusion damage in humans; and cardiovascular trauma. The beneficial effects of the compositions and methods are achieved through the use of pharmaceutical compositions that include agents that interfere with the production and/or biological activities of sphingolipids and their metabolites, particularly sphingosine (SPH) and sphingosine-1-phosphate (S-1-P). Also disclosed are methods for identifying and isolating therapeutic agents.

RELATED APPLICATIONS

[0001] This application claims priority to U.S. patent application Ser. No. 60/257,926 entitled "Compositions and Methods for the Treatment and Prevention of Cardiac and Myocardial Disorders" by Sabbadini, Roger A., filed Dec. 22, 2000.

[0002] This application is related to U.S. patent application Ser. No. _____ (attorney docket No. 078853-0302), Ser. No. _____ (attorney docket No. 078853-0304), and Ser. No. _____ (attorney docket No. 078853-0305), each entitled "Compositions and Methods for the Treatment and Prevention of Cardiovascular Diseases and Disorders, and for Identifying Agents Therapeutic Therefor" by Sabbadini, Roger A., and filed Dec. 21, 2001.

[0003] All of the preceding applications are hereby incorporated in their entirety by reference thereto.

---------- KWIC ---------

Detail Description Paragraph - DETX (279):

[0325] **Sphingosine Kinase** (SPH kinase) catalyzes the conversion of SPH to S-1-P (Rxn. #3 in FIG. 2; see also FIG. 1). A genetic **sequence** encoding human SPH-kinase has been described (Melendez et al., **Gene** 251:19-26, 2000). Three human homologs of SPH kinase (SKA, SKB and SKC) have been described (published PCT patent application WO 00/52173). Murine SPH kinase has also been described (Kohama et al., J. Biol. Chem. 273:23722-23728, 1998; and published (PCT patent application WO 99/61581). Published PCT patent application WO 99/61581 to Spiegel is stated to describe nucleic acids encoding a **sphingosine kinase**. Published PCT patent application WO 00/52173 to Munroe et al. is stated to describe nucleic acids encoding homologues of **sphingosine kinase**. Other SPH Kinases are described by Pitson et al., "Human **sphingosine kinase**: purification, molecular cloning and characterization of the native and recombinant enzymes", Biochem J. 350:429-441, 2000; and published PCT application WO 00/70028 to Pitson et al.; and Liu et al., "Molecular Cloning and Functional Characterization of a Novel Mammalian **Sphingosine Kinase** Type 2 Isoform", The Journal of Biological Chemistry, 275:19513-19520, 2000; Vadas et al., "**Sphingosine Kinase** and Uses Thereof", PCT/AU01/00539, published as WO 01/85953 on Nov. 15, 2001; Rastelli, "Novel **Sphingosine Kinases**", PCT/US01/04789, published as WO 01/60990 on Aug. 23, 2001; Allen et al., "Human **Sphingosine Kinase Gene**", PCT/EP00/09498, published as WO 01/31029 on May 3, 2001.

PGPUB-DOCUMENT-NUMBER: 20030026799

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030026799 A1

TITLE: Compositions and methods for the treatment and prevention of cardiovascular diseases and disorders, and for identifying agents therapeutic therefor

PUBLICATION-DATE: February 6, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Sabbadini, Roger A.	Lakeside	CA	US	

APPL-NO: 10/ 028156

DATE FILED: December 21, 2001

RELATED-US-APPL-DATA:

non-provisional-of-provisional 60257926 20001222 US

US-CL-CURRENT: 424/130.1, 514/12

ABSTRACT:

Methods and compositions are disclosed that are useful for the prevention and/or treatment of cardiovascular and cardiac diseases and disorders, or damage resulting from surgical or medical procedures that may cause ischemic or ischemic/reperfusion damage in humans; and cardiovascular trauma. The beneficial effects of the compositions and methods are achieved through the use of pharmaceutical compositions that include agents that interfere with the production and/or biological activities of sphingolipids and their metabolites, particularly sphingosine (SPH) and sphingosine-1-phosphate (S-1-P). Also disclosed are methods for identifying and isolating therapeutic agents.

RELATED APPLICATIONS

[0001] This application claims priority to U.S. patent application Ser. No. 60/257,926 entitled "Compositions and Methods for the Treatment and Prevention of Cardiac and Myocardial Disorders" by Sabbadini, Roger A., filed Dec. 22, 2000.

[0002] This application is related to U.S. patent application Ser. No. _____ (attorney docket No. 078853-0302), Ser. No. _____ (attorney docket No. 078853-0305), and Ser. No. _____ (attorney docket No. 078853-0306), each entitled "Compositions and Methods for the Treatment and Prevention of Cardiovascular Diseases and Disorders, and for Identifying Agents Therapeutic Therefor" by Sabbadini, Roger A., and filed Dec. 21, 2001.

[0003] All of the preceding applications are hereby incorporated in their entirety by reference thereto.

---------- KWIC ---------

Detail Description Paragraph - DETX (283):

[0329] **Sphingosine Kinase** (SPH kinase) catalyzes the conversion of SPH to S-1-P (Rxn. #3 in FIG. 2; see also FIG. 1). A genetic **sequence** encoding human SPH-kinase has been described (Melendez et al., **Gene** 251:19-26, 2000). Three human homologs of SPH kinase (SKA, SKB and SKC) have been described (published PCT patent application WO 00/52173). Murine SPH kinase has also been described (Kohama et al., J. Biol. Chem. 273:23722-23728, 1998; and published (PCT patent application WO 99/61581). Published PCT patent application WO 99/61581 to Spiegel is stated to describe nucleic acids encoding a **sphingosine kinase**. Published PCT patent application WO 00/52173 to Munroe et al. is stated to describe nucleic acids encoding homologues of **sphingosine kinase**. Other SPH Kinases are described by Pitson et al., "Human **sphingosine kinase**: purification, molecular cloning and characterization of the native and recombinant enzymes", Biochem J. 350:429-441, 2000; and published PCT application WO 00/70028 to Pitson et al.; and Liu et al., "Molecular Cloning and Functional Characterization of a Novel Mammalian **Sphingosine Kinase** Type 2 Isoform", The Journal of Biological Chemistry, 275:19513-19520, 2000; Vadas et al., "**Sphingosine Kinase** and Uses Thereof", PCT/AU01/00539, published as WO 01/85953 on Nov. 15, 2001; Rastelli, "Novel **Sphingosine Kinases**", PCT/US01/04789, published as WO 01/60990 on Aug. 23, 2001; Allen et al., "Human **Sphingosine Kinase Gene**", PCT/EP00/09498, published as WO 01/31029 on May 3, 2001.

PGPUB-DOCUMENT-NUMBER: 20020169287

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20020169287 A1

TITLE: Novel genes and their use in the modulation of obesity, diabetes and energy imbalance

PUBLICATION-DATE: November 14, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Collier, Gregory	Ocean Grove		AU	
Zimmet, Paul Zev	Toorak		AU	
Walder, Kenneth Russell	Ocean Grove		AU	
Windmill, Kelly Fiona	Newtown		AU	
McMillan, Janine Susan	Torquay		AU	

APPL-NO: 10/ 039050

DATE FILED: December 31, 2001

RELATED-US-APPL-DATA:

child 10039050 A1 20011231

parent continuation-of PCT/AU00/00786 20000629 US UNKNOWN

US-CL-CURRENT: 530/350, 435/320.1 , 435/325 , 435/69.1 , 536/23.5

ABSTRACT:

The present invention relates generally to nucleic acid molecules encoding proteins associated with the modulation of obesity, diabetes and/or metabolic energy levels. More particularly, the present invention is directed to nucleic acid molecules and the recombinant and purified proteins encoded thereby and their use in therapeutic and diagnostic protocols for conditions such as obesity, diabetes and energy imbalance. The subject nucleic acid molecules and proteins and their derivatives, homologs, analogs, chemical equivalents and mimetics are proposed as therapeutic and diagnostic agents for obesity, diabetes and energy imbalance.

---------- KWIC ---------

Detail Description Paragraph - DETX (59):

[0138] The nucleic acid molecule may be ligated to an expression vector capable of expression in a prokaryotic cell (e.g. E. coli) or a eukaryotic cell (e.g. yeast cells, fungal cells, insect cells, mammalian cells or plant cells).

The nucleic acid molecule may be ligated or fused or otherwise associated with a nucleic acid molecule encoding another entity such as, for example, a signal peptide. It may also comprise additional nucleotide **sequence** information fused, linked or otherwise associated with it either at the 3' or 5' terminal portions or at both the 3' and 5' terminal portions. The nucleic acid molecule may also be part of a vector, such as an expression vector. The latter embodiment facilitates production of recombinant forms of **sphingosine kinase** which forms are encompassed by the present invention.

PGPUB-DOCUMENT-NUMBER: 20020099029

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20020099029 A1

TITLE: Induction of blood vessel formation through administration of polynucleotides encoding sphingosine kinases

PUBLICATION-DATE: July 25, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Liau, Gene	Darnestown	MD	US	
Stefansson, Steingrimur	Gaithersburg	MD	US	
Su, Joseph	Germantown	MD	US	

APPL-NO: 09/ 970516

DATE FILED: October 4, 2001

RELATED-US-APPL-DATA:

non-provisional-of-provisional 60238230 20001005 US

US-CL-CURRENT: 514/44, 424/93.2 , 424/94.5

ABSTRACT:

A method of inducing blood vessel formation in an animal by administering to the animal a polynucleotide encoding a sphingosine kinase, or an analogue, fragment, or derivative thereof. The polynucleotide may be contained in an appropriate expression vector, such as a viral vector. The delivery of sphingosine kinase through administration of an expression vector which expresses sphingosine kinase provides for the formation of larger blood vessels containing a well defined structure that is supported by mural cells such as pericytes and smooth muscle cells.

---------- KWIC ---------

Summary of Invention Paragraph - BSTX (4):

[0003] Vascular endothelial cells undergo morphogenesis into capillary networks in response to angiogenic factors. It was shown previously that sphingosine-1-phosphate, or SPP, a platelet-derived bioactive lipid, is an important sphingolipid-derived second messenger in mammalian cells that acts to promote proliferation and to inhibit apoptosis. (Olivera, et al., Nature, Vol. 365, pgs. 557-560 (Oct. 7, 1993); Spiegel, et al., J. Leukoc. Biol., Vol. 65, No. 3, pgs. 341-344 (March 1999).) Recently, SPP was defined as a novel

regulator of angiogenesis. (Lee, et al., Cell, Vol. 99, No. 3, pgs. 301-312 (Oct. 29, 1999).) SPP activates the endothelial cell differentiation **genes** (EDG) EDG-1 and EDG-3 subtypes of G protein-coupled receptors on endothelial cells. Both EDG-1 and EDG-3 regulated signaling pathways are required for endothelial cell morphogenesis into capillary-like networks. SPP induces the Gi/mitogen-activated protein kinase cell survival pathway and enhances small GTPase Rho and Rac coupled adherens junction assembly. (Lee, 1999.) The level of SPP is regulated potentially by the enzyme that catalyzes the phosporylation of sphingosine to SPP. The cloning and characterization of the first mammalian **sphingosine kinases** (murine SPHK1.alpha. and SPHK1.beta.) has been reported. (Kohama, et al., J. Biol. Chem., Vol. 273, No. 37, pgs. 23722-23728 (Sep. 11, 1998)). Human **sphingosine kinases** (SPHK1 and SPHK2) have also been reported. (Nava, et al., FEBS, 473:81-84 (2000) and Liu, et al., J. Biol. Chem., 275:19513-19520 (2000).)

Brief Description of Drawings Paragraph - DRTX (4):

[0007] FIG. 2 shows the cDNA and amino acid **sequences** for murine **sphingosine kinase** 1.alpha..

Detail Description Paragraph - DETX (6):

[0016] In a preferred embodiment, the **sphingosine kinase,** or analogue, fragment, or derivative thereof is administered to the animal by administering to the animal an effective amount of a polynucleotide encoding a **sphingosine kinase,** or an analogue, fragment, or derivative thereof. The **sphingosine kinase** is mammalian, preferably primate, and most preferably human **sphingosine kinase**. Specific examples of **sphingosine kinase** amino acid **sequences** and the polynucleotides encoding them are found in Genbank for human SPHK1 and SPHK2 (accession numbers AF200328 and AF245447), mouse SPHK1.alpha., SPHK1.beta., and SPHK2 (accession numbers AF068748, AF068749, and AF245448), and rat SPHK1a, SPHK1c, SPHK1d, SPHK1e, and SPHK1f (accession numbers AB049571, AB049572, AB049573, AB049574, and AB049575). SEQ. ID NO:1 and SEQ ID NO:2 show the cDNA and amino acid **sequences** for human SPHK1. SEQ ID NO:3 and SEQ ID NO:4 show the cDNA and amino acid **sequences** for human SPHK2. An analogue of **sphingosine kinase** includes, but is not limited to, splice variants of **sphingosine kinase,** deletions in the coding region, and multiple forms (T. Kohama et al., JBC, 273:23722-23728 (1998); H. Liu et al., JBC, 275:19513-19520 (2000); Y. Banno, et al., Biochem J., 335:301-304 (1998)). A fragment of **sphingosine kinase** is a portion of the protein that retains its activity for inducing blood vessel formation. A derivative of **sphingosine kinase** includes, but is not limited to, modifications to alter **sphingosine kinase** regulation or biological activity. Non-limiting examples include the addition of a signal **sequence** to force secretion of the enzyme or modification of the calcium, calmodulin binding domain, ATP binding site, or membrane retention **sequences**. The polynucleotide is under the control of a suitable promoter. It is to be understood, however, that the scope of the present invention is not to be limited to any specific promoters.

Detail Description Paragraph - DETX (7):

[0017] Preferably, the polynucleotide encoding the **sphingosine kinase,** or an

analogue, fragment, or derivative thereof is contained in an appropriate expression vehicle. Such expression vehicles include, but are not limited to, plasmids, eukaryotic vectors, prokaryotic vectors (such as, for example, bacterial vectors), and viral vectors. hi one embodiment, the vector is a viral vector. Viral vectors which may be employed include RNA virus vectors (such as retroviral vectors, including lentiviral vectors) and DNA virus vectors (such as adenoviral vectors, adeno-associated virus vectors, Herpes Virus vectors, and vaccinia virus vectors). When a DNA virus vector is employed in constructing the vector, the polynucleotide encoding the **sphingosine kinase** is in the form of DNA. When an RNA virus vector is employed in constructing the vector, the polynucleotide encoding the **sphingosine kinase** is in the form of RNA. Preferable viral vectors include adenoviral vectors (preferably lacking all viral **genes,** i.e. high capacity or gutless), lentiviral vectors (e.g. HIV, BIV-based), and adeno-associated virus (AAV) vectors.

Detail Description Paragraph - DETX (10):

[0020] In a preferred embodiment, the adenoviral vector comprises an adenoviral 5' ITR; an adenoviral 3' ITR; an adenoviral encapsidation signal; a DNA **sequence** encoding a **sphingosine kinase,** or an analogue, fragment, or derivative thereof, and a promoter controlling the DNA **sequence** encoding a **sphingosine kinase,** or an analogous, fragment, or derivative thereof. The vector is free of at least the majority of adenoviral E1 and E3 DNA **sequences,** but is not free of all of the E2 and E4 DNA **sequences,** and DNA **sequences** encoding adenoviral proteins promoted by the adenoviral major late promoter. In one embodiment, the vector also is free of at least a portion of at least one DNA **sequence** selected from the group consisting of the E2 and E4 DNA **sequences**.

Detail Description Paragraph - DETX (13):

[0023] Such a vector, in a preferred embodiment, is constructed first by constructing, according to standard techniques, a shuttle plasmid which contains, beginning at the 5' end, the "critical left end elements," which include an adenoviral 5' ITR, an adenoviral encapsidation signal, and an E1a enhancer **sequence**; a promoter (which may be an adenoviral promoter or a foreign promoter); a multiple cloning site (which may be as herein described); a poly A signal; and a DNA segment which corresponds to a segment of the adenoviral genome. The promoter may, in one embodiment, be a regulatable promoter, such as, for example, a glucocorticoid-responsive promoter or an estrogen-responsive promoter, or the promoter may be a tissue--specific promoter. The vector also may, in another embodiment, contain genomic elements which may increase and/or maintain expression of the DNA **sequence** encoding a **sphingosine kinase,** or an analogue, fragment, or derivative thereof. Such genomic elements include, but are not limited to, introns, exons, polyadenylation **sequences,** and 5' and 3' untranslated regions. Such genomic elements, and representative examples thereof, also are described in U.S. Pat. No. 5,935,935, issued Aug. 10, 1999. The vector also may contain a tripartite leader **sequence**. The DNA segment which corresponds to a segment of the adenoviral genome serves as a substrate for homologous recombination with an adenovirus. The plasmid may also include a selectable marker and an origin of replication. The origin of replication may be a bacterial origin of replication. Representative examples

of such shuttle plasmids include pAvS6, which is described in published PCT Application Nos. WO 94/23582, published Oct. 27, 1994, and WO 95/09654, published Apr. 13, 1995, and in U.S. Pat. No. 5,543,328, issued Aug. 6, 1996. The DNA **sequence** encoding a **sphing sine kinase,** or an analogue, fragment, or derivative thereof then may be inserted into the multiple cloning site of the shuttle plasmid to produce a plasmid vector.

Detail Description Paragraph - DETX (16):

[0026] Through such homologous recombination, a vector is formed which includes an adenoviral 5' ITR, an adenoviral encapsidation signal; an E1a enhancer **sequence**; a promoter; a DNA **sequence** encoding a **sphingosine kinase,** or an analogue, fragment, or derivative thereof; a poly A signal; adenoviral DNA **sequences**; and an adenoviral 3' ITR. The vector also may include a tripartite leader **sequence**. The vector may then be transfected into a helper cell line, such as the 293 helper cell line (ATCC No. CRL1573), which will include the E1a and the E1b DNA **sequences,** which are necessary for viral replication, and to generate adenoviral particles. Transfection may take place by electroporation, calcium phosphate precipitation, microinjection, or through proteoliposomes.

Detail Description Paragraph - DETX (24):

[0034] In another embodiment, the adenoviral vector is free of all adenoviral coding regions. This "gutless" adenoviral vector includes an adenoviral 5' ITR, an adenoviral packaging signal, a DNA **sequence** encoding **sphingosine kinase** or an analogue, fragment, or derivative thereof, and an adenoviral 3' ITR. The vector contains from about 26 kb to about 38 kb, preferably 28 kb to 32 kb, and may include one or more genomic elements.

Detail Description Paragraph - DETX (25):

[0035] The various adenoviral vectors may include promoters other than a **sphingosine kinase** promoter, such as tissue-specific promoters. The vector also may include, in addition to a DNA **sequence** encoding a **sphingosine kinase,** or an analogue, fragment, or derivative thereof, DNA **sequences** encoding additional proteins which facilitate the generation of new blood vessels, such as, but not limited to, vascular endothelial growth factors (VEGFs), fibroblast growth factors (FGFs), IGFs, angiopoietins, including angiopoietin 1, and angiopoietin 2, TGF-.beta., hypoxia inducible factors (HIFs) such as HIF1-.alpha., monocyte chemoattractant proteins (MCPs) such as MCP-1, nitric oxide synthase, ephrins, such as ephrin B2, and other angiogenic **genes,** platelet derived endothelial growth factor, and Interleukin-8.

Detail Description Paragraph - DETX (42):

[0052] The retroviral plasmid vector including the polynucleotide encoding a **sphingosine kinase,** or an analogue, fragment, or derivative thereof is transduced into a packaging cell line including nucleic acid **sequences** encoding the gag, pol, and env retroviral proteins. Examples of such packaging cell lines include, but are not limited to, the PE501, PA317 (ATCC No. CRL 9078), .PSI.-2, .PSI.-AM, PA12, T19-14X, VT-19-17-H2, .PSI.CRE, .PSI.CRIP, GP+E-86, GP+envAm12, and DAN cell lines as described in Miller, Human **Gene** Therapy, Vol.

1, pgs. 5-14 (1990), which is incorporated herein by reference in its entirety, or the 293T cell line (U.S. Pat. No, 5,952,225). The vector may transduce the packaging cells through any means known in the art. Such means include, but are not limited to, electroporation, the use of liposomes, and CaPO.sub.4 precipitation. Such producer cells generate infectious retroviral vector particles which include the polynucleotide encoding a **sphingosine kinase,** or an analogue, fragment, or derivative thereof.

Detail Description Paragraph - DETX (47):

[0057] Another embodiment has the expression of **sphingosine kinase** controlled by an inducible promoter. The use of an inducible **gene** expression system would allow the precise regulation of **sphingosine kinase** in a reversible manner. Several inducible systems are currently available. One example of a controlled promoter system in the Tet-On.TM. and Tet-Off.TM. systems currently available from Clontech (Palo Alto, Calif.). Tet-Off.TM. system uses the tetracycline-controlled transactivator (tTA), which is composed of the tet repressor protein (TetR) and the VP16 activation domain. tTA activates transcription in the absence of tetracycline. The Tet-On.TM. system uses the reverse tetracycline-controlled transactivator (rtTA) and activates transcription in the presence of tetracycline. Both systems use the tetracycline-response element (TRE), which contains 7 repeats of the tet operator **sequence,** and the target **gene, such as sphingosine kinase**. tTA or rtTA bind to the TRE, activating transcription of the target **gene**. This promoter system allows the regulated expression of the transgene controlled by tetracycline or tetracycline derivatives, such as doxycycline. This system could be used to control the expression of **sphingosine kinase** in this instant invention.

Detail Description Paragraph - DETX (57):

[0065] The plasmid pCR3.1sphK1.alpha., derived from pCR3.1 (Invitrogen), was obtained from Thomas Baumruker (Novartis, Vienna, Austria) and contains the mouse **sphingosine kinase** alpha cDNA. pCR3.1sphK1x was digested with HindIII and NotI to isolate a 1,531 bp insert containing the coding **sequence** for sphK1.alpha.. The fragment was blunt-ended and cloned into the EcoRV site of pAVS6a1x, an adenoviral shuttle plasmid containing a lox recombination site, to create pAV1xsphK1.alpha. (FIG. 1). pAVS6a1x had been formed by adding a lox site to pAVS6a (U.S. Pat. No. 5,543,328). A 535 bp ClaI/NcoI fragment from pAVH8-101 1x, containing the SV40 polyA signal and lox site was inserted into pAVS6a digested with ClaI and NcoI and linearized (4,745 bp). The sphK1.alpha. cDNA was cloned downstream of the RSV promoter and the adenoviral tripartite leader **sequence,** and included the SV40 polyadenylation signal and a homologous recombination region. A large-scale plasmid preparation was prepared using the alkaline lysis method and purified using a CsTFA gradient following standard protocols. The cDNA then was **sequenced**. The sphK1.alpha. coding **sequence** is 1,149 bp (SEQ ID NO:5) and encodes a 382 amino acid protein (SEQ ID NO:6). The cDNA and amino acid **sequences** are shown in FIG. 2.

Detail Description Paragraph - DETX (79):

[0084] Overexpression of **Sphing sine Kinase** protects cardiomyocytes from

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TITLE: Novel sphingosine kinases

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ABSTRACT:

Disclosed herein are novel human nucleic acid sequences that encode polypeptides. Also disclosed are polypeptides encoded by these nucleic acid sequences, and antibodies that immunospecifically-bind to the polypeptide, as well as derivatives, variants, mutants, or fragments of the aforementioned polypeptide, polynucleotide, or antibody. The invention further discloses therapeutic, diagnostic and research methods for diagnosis, treatment, and prevention of disorders involving any one of these novel human nucleic acids and proteins.

RELATED APPLICATIONS

[0001] This application claims priority to U.S. provisional applications Ser. No.60/182,360, filed Feb. 14, 2000, and Ser. No. 60/191,261, filed Mar. 22, 2000, which are incorporated herein by reference in their entirety.

---------- KWIC ---------

Detail Description Paragraph - DETX (2):

[0031] The invention is based, in part, upon the discovery of novel nucleic

acid **sequences** that encode novel polypeptides, particularly **sphingosine kinases**. The nucleic acids, and their encoded polypeptides, are collectively designated herein as "SphK".

Claims Text - CLTX (54):

53. A non-human transgenic animal comprising a transgene disrupting an endogenous **sphingosine kinase gene**.

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PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20020042358 A1

TITLE: Sphingosine kinase, cloning, expression and methods of use

PUBLICATION-DATE: April 11, 2002

INVENTOR-INFORMATION:

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RELATED-US-APPL-DATA:

non-provisional-of-provisional 60186352 20000302 US

US-CL-CURRENT: 514/1, 435/194 , 435/325 , 435/4 , 536/23.2

ABSTRACT:

The invention provides molecules that encode sphingosine kinase, the enzyme that catalyzes the phosphorylation of sphingosine to form sphingosine-1-phosphate (SPP). Vectors and host cells which express sphingosine kinase are also provided, as are methods for evaluating the stimulatory or inhibitory effects of agents on sphingosine kinase production and activity.

[0001] This application takes priority from Provisional Patent Application 60/186,352, which was filed Mar. 3, 2000 and is also a continuation-in-part of U.S. Ser. No. 09/530,868, which was filed on May 5, 2000 and was filed under rule 371 as the U.S. application from PCT/US99/11521.

---------- KWIC ---------

Summary of Invention Paragraph - BSTX (4):

[0003] Sphingosine-1-phosphate (SPP), a sphingolipid metabolite, which regulates diverse biological processes, such as cell growth, differentiation, survival, motility, and calcium mobilization, is now emerging as a new member of a class of lipid signaling molecules with dual intra and intercellular actions. This phosphorylated derivative of sphingosine, the structural backbone of all sphingolipids, has many of the hallmarks of classical second messengers. The level of SPP is very low in cells and is rapidly increased by

activation of **sphingosine kinase** (SPHK), the enzyme responsible for the formation of SPP. SPHK is a member of a highly conserved **gene** family and is distinct from other known lipid kinases. Changes in SPHK activity is induced by diverse physiological stimuli, including platelet-derived growth factor (PDGF), nerve growth factor (NGF), muscarinic acetylcholine agonists; TNF-a, activation of protein kinase C (PKC), and cross-linking of the immunoglobulin receptors. Like to other signaling molecules, SPP has a short half life due to rapid turnover catalyzed by SPP lyase and/or SPP phosphatase. Inhibition of SPP formation by competitive inhibitors of SPHK blunted the mitogenic response to PDGF, the cytoprotective effects of NGF, vitamin D3, PKC, and cAMP activators. Furthermore, calcium mobilization induced by FceR1, FcgR1, and muscarinic acetylcholine receptors are affected by SPP formation.

Brief Description of Drawings Paragraph - DRTX (2):

[0007] FIG. 1 shows predicted amino acid **sequences** of two murine **sphingosine kinases** (SEQ ID NO:1 and SEQ ID NO:2).

Detail Description Paragraph - DETX (15):

[0023] Purified **sphingosine kinase** was electrophoresed on SDS-PAGE and the Coomassiestained 49 kd band excised. After S-carboxy-amidomethylation, this band was subjected to in-gel tryptic digestion as described. The resulting peptide mixture was separated by microbore high performance liquid chromatography on a Zorbax C18 1.0 mm by 150-mm reverse-phase column in a Hewlett-Packard 1090 HPLC with a1040 diode array detector. Fractions were selected for sequencing based on differential UV absorbance at 205, 277, and 292 nm, and the peptide **sequences** were determined by automated Edman degradation. Complementary peptide **sequence** information was also obtained on 10% of the digest mixture by collisionally induced dissociation using microcapillary HPLC electrospray ionization/tandem mass spectrometry on a Finnigan TSQ7000 triple quadruple mass spectrometer. **Sequences** of smaller peaks were determined on an Applied Biosystems Procise cLC 494 **sequencer** or by microcapillary HPLC-MS as above or on a Finnigan LaserMat 2000 Time-of-Flight Mass Spectrometer Matrix-assisted Laser Desorption TOF/MS (MALDI-TOFMS).

Detail Description Paragraph - DETX (17):

[0025] The 49 kD **sphingosine kinase** polypeptide, purified from rat kidney, was excised from an SDS gel and subjected to trypsin digestion. The resulting peptides were separated by microcapillary reverse-phase HPLC and **sequences** of 8 peptides were determined by Edman degradation or MALDI mass spectrometry.

Detail Description Paragraph - DETX (18):

[0026] Homology searches (BLAST) against a comprehensive nonredundant database revealed no matches to known proteins. However, when the database of expressed **sequence** tags (dbEST) at NCBI was searched using the tBLASTn algorithm, an EST (Genbank accession number AA011725) containing **sequences** homologous to 3 of the 8 peptides (peptides 5, 2, and 4) was retrieved. A further search with peptides 1, 3 and 7 yielded 4 additional ESTs (Genbank accession numbers AA000819, AA107451, AA592274, and AA389543). The nucleotide

sequences of mouse ESTs AA000819 and AA592274 were then used to search dbEST to obtain EST AA389187. Clones AA107451 and AA389187 were highly homologous at their 3 ends, but were slightly divergent at their 5' ends. sequencing of the full-length cDNAs revealed apparent open reading frames coding for 381 and 388 amino acid polypeptides containing **sequences** highly homologous to seven isolated peptides distributed throughout the protein, and these are thus designated SPHK1a and SPHK1b. In addition, both contained a portion of peptide 8. SPHK1a and 1b have predicted pIs of 6.68 and 6.89 and MWs of 42344 and 43254, respectively, in agreement with the MW of purified rat kidney **sphingosine kinase**. Because SPHK1b only differs by a few amino acids at the N terminus, it may arise by alternative splicing. However, both **sequences** lacked Kozak concensus **sequences,** suggesting that these cDNAs may not include the actual initiation **sequences**.

Detail Description Paragraph - DETX (19):

[0027] SPHK1a has 2 overlapping calcium/calmodulin binding concensus **sequences** of the 1-8-14 Type B motif ((FILVW)xxx-xxx(FAILVW)xxxxx(FILVW), containing net positive charges of 2-4). In addition, near the C-terminus, SPHK1a contains 2 overlapping calcium/calmodulin binding consensus **sequences,** one of Type B and one of Type A ((FILVW)xxx(FAILVW)xx(FAILVW)xxxxx(FILVW)) containing net positive charges of 3-6). SPHK1b contains all the above calcium/calmodulin binding consensus **sequences** as well as an additional Type B motif at the N-terminus. The existence of multiple calcium/calmodulin binding motifs supports previous observations that purified rat kidney **sphingosine kinase** binds tightly to calmodulin-sepharose in the presence of calcium.

Detail Description Paragraph - DETX (20):

[0028] Analysis of the domain structure of SPHK1a obtained by searching the protein data base (GenBank and Prosite) revealed several putative post-translational phosphorylation motifs: one kinase A, two casein kinase II, and eight protein kinase C phosphorylation sites. Interestingly, it was previously demonstrated that inhibition of ceramide-induced apoptosis by protein kinase C activation results from stimulation of **sphingosine kinase** and concomitant increase in cellular SPP levels. **Sphingosine kinase** is thought to be mainly a cytosolic enzyme. Consistent with this, a hydropathy plot indicates that SPHK1a does not contain signal peptide or hydrophobic transmembrane **sequences** (data not shown).

Detail Description Paragraph - DETX (22):

[0030] BLAST searches using mSPHK1a **sequences** identified an EST clone (AA026479) which contained **sequences** homologous to several conserved domains of mSPHK. Human **sphingosine kinase** 1 was cloned by RT-CPR using poly(A).sup.+ RNA from HEK293 cells and a **gene**-specific antisense primer hspk1-ASP1:5.degree.-ACCATTGTCCAGTGAG The cDNA was extended by 5'RACE (Life Technologies) with two consecutive PCR (polymerase chain reaction) reactions using LA Taq (TaKaRa). First PCTR:5'RACE (rapid amplification of cDNA ends) Abridged Anchor Primer and the antisense primer hspk1-GSP2, 5'-TTCCTACAGGGAGGTAGGCC at 94.degree. C. for 2 minutes followed by 30 cycles of amplification (94.degree. C. for 1 minute, 55.degree. C. for 1 minute,

72.degree. C. for 2 minutes) and primer extension at 72.degree. C. for 5 minutes. Second PCR:Abridged Universal Amplification Primer and the antisense primer hspkl-GSP 3,5'-GGCTGCCAGACGCAGGAAGG using a program similar to the first PCR but with annealing at 65.degree. C. The PCR products were cloned into pCR2.1 (TA cloning, Invitrogen) and **sequences** confirmed by automated sequencing. To make expression constructs, a primer set was designated as follows: sense primer containing Kozak **sequence** and ATG start codon, sphk1-GSP4 (5'-GCCACCATGGATCCAGCGGGCGGCCCC)- ; antisense primer, sphk1-GSP5 (5'TCATAAGGGCTCTTCTGGCGGTGGCATCTG). The PCR reaction was performed using human
fetus Marathon-Ready cDNA (Clontech) as template with the above primers, and the amplification product was subcloned into pCR3.1 (Eukaryotic TA Cloning, Invitrogen). In addition, hSPHK1 was tagged at the N-terminus by subcloning into a pcDNA-c-myc vector using high fidelity taq polymerase (Pfu, Stratagene). hSPHK1 accession number is AF238083.

Claims Text - CLTX (6):

5. An isolated and purified DNA fragment of claim 2 which encodes a peptide of murine **sphingosine kinase,** said DNA fragment comprising the **sequence** specified in Genbank Accession no. AF068748, or GenBank Accession no. AF068749, or a polynucleotide fragment of said **sequence** comprising at least 30 nucleotides.

Claims Text - CLTX (23):

22. A diagnostic kit for detecting **sphingosine kinase** RNA/cDNA in a sample comprising primers or oligonucleotides specific for **sphingosine kinase** RNA or CDNA suitable for hybridization to **sphingosine kinase** RNA or CDNA and/or amplification of **sphingosine kinase sequences** and suitable ancillary reagents.

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TITLE: Mammalian sphingosine kinase type 2 isoforms, cloning, expression and methods of use thereof

PUBLICATION-DATE: April 11, 2002

INVENTOR-INFORMATION:

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ABSTRACT:

Nucleic acids encoding mouse and human sphingosine kinase type 2 isoforms, methods for detecting agents or drugs which inhibit or promote sphingosine activity and therapeutic agents containing peptides or antibodies to peptides encoded by such nucleic acids.

CROSS-REFERENCE TO RELATED APPLICATION

[0001] The present application claims the benefit of Provisional Application Serial No. 60/194,318, filed Apr. 3, 2000, wherein priority under 35 USC 119(e) is claimed.

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Summary of Invention Paragraph - BSTX (22):

[0022] The present invention is also directed to an isolated and purified DNA which encodes a peptide of a **sphingosine kinase** type 2 isoform, the DNA comprising a **sequence** selected from the group consisting of the **sequence** of Genbank Accession No. bankit325787 and the **sequence** of Genbank Accession No. bankit325752.

Summary of Invention Paragraph - BSTX (37):
[0037] The present invention is additionally directed to a diagnostic kit for detecting **sphingosine kinase** type 2 RNA/cDNA in a sample comprising primers or oligonucleotides specific for **sphingosine kinase** type 2 RNA or cDNA suitable for hybridization to **sphingosine kinase** type 2 RNA or cDNA and/or amplification of **sphingosine kinase** type 2 **sequences** and suitable ancillary reagents.

Summary of Invention Paragraph - BSTX (38):
[0038] **Sphingosine kinase** catalyzes the phosphorylation of sphingosine to yield SPP. Based on **sequence** homology to murine and human **sphingosine kinase**-1 (SPHK1), which was recently cloned (Kohama, et al., J. Biol. Chem., 273, 23722-23728, (1998)), the present invention is directed to the cloning, functional characterization, and tissue distribution of a second type of mouse and human **sphingosine kinase** (mSPHK2 and hSPHK2).

Brief Description of Drawings Paragraph - DRTX (3):
[0043] FIG. 1A shows predicted amino acid **sequences** of murine and human type 2 **sphingosine kinase** based on non-ClustalW alignment of the predicted amino acid **sequences** of ("mSPHK2") and human **sphingosine kinase** 2 ("hSPHK2"). Identical and conserved amino acid substitutions are shaded dark and light gray, respectively. The dashes represent gaps in **sequences** and numbers on the right refer to the amino acid **sequence** of mSPHK2. The conserved domains (C1 to C5) are indicated by lines.

Detail Description Paragraph - DETX (3):
[0067] In addition, isolated nucleic acid molecules of the invention include DNA molecules which comprise **sequences** substantially different from those described above but which, due to the degeneracy of the genetic code, still encode mammalian **sphingosine kinase** type 2 isoforms. Of course, the genetic code and species-specific codon preferences are well known in the art. Thus, it would be routine for one of ordinary skill in the art to generate the degenerate variants described above, for instance, to optimize codon expression for a particular host (e.g., change codons in the human mRNA to those preferred by a bacterial host such as E. coli).

Detail Description Paragraph - DETX (10):
[0074] The present invention further relates to variants of the nucleic acid molecules of the present invention, which encode portions, analogs or derivatives of the **sphingosine kinase** type 2 isoform polypeptides shown in FIG. 1A. Variants may occur naturally, such as a natural allelic variant. By an "allelic variant" is intended one of several alternate forms of a **gene** occupying a given locus of a chromosome of an organism. Non-naturally occurring variants may be produced by known mutagenesis techniques. Such variants include those produced by nucleotide substitution, deletion or addition of one or more nucleotides in the coding or noncoding regions or both. Alterations in the coding regions may produce conservative or nonconservative amino acid substitutions, deletions, or additions. Especially preferred among

these are silent substitutions, additions, and deletions which do not alter the properties and activities of **sphingosine kinase** type 2 isoform polypeptides disclosed herein or portions thereof. Also preferred in this regard are conservative substitutions.

Detail Description Paragraph - DETX (29):

[0093] Polynucleotide probes for the detection of **sphingosine kinase** type 2 RNA can be designed from the **sequence** available at accession numbers AF068748 and/or AF068749 for the mouse **sequence** (Kohama, T., et al., J. Biol. Chem., 273:23722-23728). For example, RNA isolated from samples can be coated onto a surface such as a nitrocellulose membrane and prepared for northern hybridization. In the case of in situ hybridization of biopsy samples, for example, the tissue sample can be prepared for hybridization by standard methods known in the art and hybridized with polynucleotide **sequences** which specifically recognize **sphingosine kinase** type 2 RNA. The presence of a hybrid formed between the sample RNA and the polynucleotide can be detected by any method known in the art such as radiochemistry, or immunochemistry, to name a few.

Detail Description Paragraph - DETX (31):

[0095] The DNA **sequence of sphingosine kinase** type 2 can be used to design primers for use in the detection of **sphingosine kinase** type 2 using the polymerase chain reaction (PCR) or reverse transcription PCR (RT-PCR). The primers can specifically bind to the **sphingosine kinase** type 2 cDNA produced by reverse transcription of **sphingosine kinase** type 2 RNA, for the purpose of detecting the presence, absence, or quantifying the amount of **sphingosine kinase** type 2 by comparison to a standard. The primers can be any length ranging from 7 to 40 nucleotides, preferably 10 to 35 nucleotides, most preferably 18 to 25 nucleotides homologous or complementary to a region of the **sphingosine kinase** type 2 **sequence**.

Detail Description Paragraph - DETX (32):

[0096] Reagents and controls necessary for PCR or RT-PCR reactions are well-known in the art. The amplified products can then be analyzed for the presence or absence of **sphingosine kinase** type 2 **sequences**, for example, by gel fractionation, by radiochemistry, and immunochemical techniques. This method is advantageous, since it requires a small number of cells. Once **sphingosine kinase** type 2 is detected, a determination of whether the cell is overexpressing or underexpressing **sphingosine kinase** type 2 can be made by comparison to the results obtained from a normal cell using the same method. Increased **sphingosine kinase** type 2 RNA levels correlate with increased cell proliferation and reduced cell death.

Detail Description Paragraph - DETX (50):

[0114] Agents which decrease the level of **sphingosine kinase** type 2 (i.e., in a human or an animal) or reduce or inhibit **sphingosine kinase** type 2 activity may be used in the therapy of any disease associated with the elevated levels of **sphingosine kinase** type 2 or diseases associated with increased cell

proliferation, such as cancer. An increase in the level of **sphingosine kinase** type 2 is determined when the level of **sphing sine kinase** type 2 in a tumor cell is about 2 to 3 times the level of **sphing sine kinase** type 2 in the normal cell, up to about 10 to 100 times the amount of **sphing sine kinase** type 2 in a normal cell. Agents which decrease **sphing sine kinase** type 2 RNA include, but are not limited to, one or more ribozymes capable of digesting **sphingosine kinase** type 2 RNA, or antisense oligonucleotides capable of hybridizing to **sphingosine kinase** type 2 RNA, such that the translation of **sphingosine kinase** type 2 is inhibited or reduced resulting in a decrease in the level of **sphingosine kinase** type 2. These antisense oligonucleotides can be administered as DNA, as DNA entrapped in proteoliposomes containing viral envelope receptor proteins (Kanoda, Y. et al., (1989), Science, 5, 243, 375) or as part of a vector which can be expressed in the target cell such that the antisense DNA or RNA is made. Vectors which are expressed in particular cell types are known in the art, for example, for the mammary gland. See Furth, J. Mammary Gland Biol. Neopl., 2, (1997), 373, for examples of conditional control of **gene** expression in the mammary gland.

Detail Description Paragraph - DETX (83):

[0140] Blast searches of the EST data base identified several ESTs that displayed significant homology to the recently cloned mSPHK1a **sequence**. Specific primers were designed from the **sequences** of these ESTs and were used to clone a new type of mouse and human **sphingosine kinase** (named mSPHK2 and hSPHK2) by the approaches of PCR cloning from a mouse brain cDNA library and 5'-RACE PCR.

Detail Description Table CWU - DETL (5):

Val Pro Leu Ser Gly Asp Gln Glu Ile Thr Pr #o Glu Leu Leu Pro Arg 130 # 135 # 140 Lys Pro Arg Leu Leu Ile Leu Val Asn Pro Ph #e Gly Gly Arg Gly Leu 145 1 #50 1 #55 1 #60 Ala Trp Gln Arg Cys Met Asp His Val Val Pr #o Met Ile Ser Glu Ala 165 # 170 # 175 Gly Leu Ser Phe Asn Leu Ile Gln Thr Glu Ar #g Gln Asn His Ala Arg 180 # 185 # 190 Glu Leu Val Gln Gly Leu Ser Leu Ser Glu Tr #p Glu Gly Ile Val Thr 195 # 200 # 205 Val Ser Gly Asp Gly Leu Leu Tyr Glu Val Le #u Asn Gly Leu Leu Asp 210 # 215 # 220 Arg Pro Asp Trp Glu Asp Ala Val Arg Met Pr #o Ile Gly Val Leu Pro 225 2 #30 2 #35 2 #40 Cys Gly Ser Gly Asn Ala Leu Ala Gly Ala Va #l Ser His His Gly Gly 245 # 250 # 255 Phe Glu Gln Val Val Gly Val Asp Leu Leu Le #u Asn Cys Ser Leu Leu 260 # 265 # 270 Leu Cys Arg Gly Gly Ser His Pro Leu Asp Le #u Leu Ser Val Thr Leu 275 # 280 # 285 Ala Ser Gly Ser Arg Cys Phe Ser Phe Leu Se #r Val Ala Trp Gly Phe 290 # 295 # 300 Leu Ser Asp Val Asp Ile His Ser Glu Arg Ph #e Arg Ala Leu Gly Ser 305 3 #10 3 #15 3 #20 Ala Arg Phe Thr Leu Gly Ala Val Leu Gly Le #u Ala Ser Leu His Thr 325 # 330 # 335 Tyr Arg Gly Arg Leu Ser Tyr Leu Pro Ala Th #r Thr Glu Pro Ala Leu 340 # 345 # 350 Pro Ile Pro Gly His Ser Leu Pro Arg Ala Ly #s Ser Glu Leu Val Leu 355 # 360 # 365 Ala Pro Ala Pro Ala Pro Ala Ala Thr His Se #r Pro Leu His Arg Ser 370 # 375 # 380 Val Ser Asp Leu Pro Leu Pro Leu Pro Gln Pr #o Ala Leu Val Ser Pro 385 3 #90 3 #95 4 #00 Gly Ser Pro Glu Pro Leu Pro Asp Leu Ser Le #u Asn Gly Gly Gly Pro 405 # 410 # 415 Glu Leu Thr Gly Asp Trp Gly Gly Ala Gly As #p Ala Pro Leu Ser Pro 420 # 425 # 430 Asp Pro Leu Leu Pro Ser Ser Pro Asn Ala Le #u Lys Thr Ala Gln Leu 435 # 440 # 445 Ser Pro Ile Ala Glu Gly Pro Pro Glu

Met Pr #o Ala Ser Ser Gly Phe 450 # 455 # 460 Leu Pro Pro Thr His Ser Ala Pro Glu Ala Se #r Thr Trp Gly Pro Val 465 4 #70 4 #75 4 #80 Asp His Leu Leu Pro Pro Leu Gly Ser Pro Le #u Pro Gln Asp Trp Val 485 # 490 # 495 Thr Ile Glu Gly Glu Phe Val Leu Met Leu Gl #y Ile Leu Thr Ser His 500 # 505 # 510 Leu Cys Ala Asp Leu Met Ala Ala Pro His Al #a Arg Phe Asp Asp Gly 515 # 520 # 525 Val Val His Leu Cys Trp Val Arg Ser Gly Il #e Ser Arg Ala Ala Leu 530 # 535 # 540 Leu Arg Ile Phe Leu Ala Met Glu His Gly As #n His Phe Ser Leu Gly 545 5 #50 5 #55 5 #60 Cys Pro His Leu Gly Tyr Ala Ala Ala Arg Al #a Phe Arg Leu Glu Pro 565 # 570 # 575 Leu Thr Pro Arg Gly Leu Leu Thr Val Asp Gl #y Glu Leu Val Glu Tyr 580 # 585 # 590 Gly Pro Ile Gln Ala Gln Val His Pro Gly Le #u Ala Thr Leu Leu Thr 595 # 600 # 605 Gly Pro Ala Gly Gln Lys Pro Gln Ala 610 # 615 <210> SEQ ID NO 13 <211> LENGTH: 2380 <212> TYPE: DNA <213> ORGANISM: Homo sapiens <220> FEATURE: <221> NAME/KEY: CDS <222> LOCATION: (7)..(1860) <300> PUBLICATION INFORMATION: <302> TITLE: Molecular cloning and functional char #acterization of a novel mammalian **sphingosine kinase** t #ype 2 isoform <303> JOURNAL: J. Biol. Chem. <304> VOLUME: 275 <305> ISSUE: 26 <306> PAGES: 19513-19520 <307> DATE: 2000-06-30 <308> DATABASE ACCESSION NUMBER: AF245447 <309> DATABASE ENTRY DATE: 2000-06-27 <400> **SEQUENCE**: 13 gccacc atg gcc ccg ccc cca ccg cca ctg gct #gcc agc acc ccg ctc 48 Met Ala Pro Pro Pro Pro Pr #o Leu Ala Ala Ser Thr Pro Leu 1 # 5 # 10 ctc cat ggc gag ttt ggc tcc tac cca gcc cg #a ggc cca cgc ttt gcc 96 Leu His Gly Glu Phe Gly Ser Tyr Pro Ala Ar #g Gly Pro Arg Phe Ala 15 # 20 # 25 # 30 ctc acc ctt aca tcg cag gcc ctg cac ata ca #g cgg ctg cgc ccc aaa 144 Leu Thr Leu Thr Ser Gln Ala Leu His Ile Gl #n Arg Leu Arg Pro Lys 35 # 40 # 45 cct gaa gcc agg ccc cgg ggt ggc ctg gtc cc #g ttg gcc gag gtc tca 192 Pro Glu Ala Arg Pro Arg Gly Gly Leu Val Pr #o Leu Ala Glu Val Ser 50 # 55 # 60 ggc tgc tgc acc ctg cga agc cgc agc ccc tc #a gac tca gcg gcc tac 240 Gly Cys Cys Thr Leu Arg Ser Arg Ser Pro Se #r Asp Ser Ala Ala Tyr 65 # 70 # 75

Detail Description Table CWU - DETL (8):

#a Glu Val Ser Gly Cys 50 # 55 # 60 Cys Thr Leu Arg Ser Arg Ser Pro Ser Asp Se #r Ala Ala Tyr Phe Cys 65 # 70 # 75 # 80 Ile Tyr Thr Tyr Pro Arg Gly Arg Arg Gly Al #a Arg Arg Arg Ala Thr 85 # 90 # 95 Arg Thr Phe Arg Ala Asp Gly Ala Ala Thr Ty #r Glu Glu Asn Arg Ala 100 # 105 # 110 Glu Ala Gln Arg Trp Ala Thr Ala Leu Thr Cy #s Leu Leu Arg Gly Leu 115 # 120 # 125 Pro Leu Pro Gly Asp Gly Glu Ile Thr Pro As #p Leu Leu Pro Arg Pro 130 # 135 # 140 Pro Arg Leu Leu Leu Leu Val Asn Pro Phe Gl #y Gly Arg Gly Leu Ala 145 1 #50 1 #55 1 #60 Trp Gln Trp Cys Lys Asn His Val Leu Pro Me #t Ile Ser Glu Ala Gly 165 # 170 # 175 Leu Ser Phe Asn Leu Ile Gln Thr Glu Arg Gl #n Asn His Ala Arg Glu 180 # 185 # 190 Leu Val Gln Gly Leu Ser Leu Ser Glu Trp As #p Gly Ile Val Thr Val 195 # 200 # 205 Ser Gly Asp Gly Leu Leu His Glu Val Leu As #n Gly Leu Leu Asp Arg 210 # 215 # 220 Pro Asp Trp Glu Glu Ala Val Lys Met Pro Va #l Gly Ile Leu Pro Cys 225 2 #30 2 #35 2 #40 Gly Ser Gly Asn Ala Leu Ala Gly Ala Val As #n Gln His Gly Gly Phe 245 # 250 # 255 Glu Pro Ala Leu Gly Leu Asp Leu Leu Leu As #n Cys Ser Leu Leu Leu 260 # 265 # 270 Cys Arg Gly Gly Gly His Pro Leu Asp Leu Le #u Ser Val Thr Leu Ala 275 # 280 # 285 Ser Gly Ser Arg Cys Phe Ser Phe Leu Ser Va #l Ala Trp Gly Phe Val 290 # 295 # 300 Ser Asp Val Asp Ile Gln Ser Glu Arg Phe Ar #g Ala Leu Gly Ser Ala 305 3 #10 3 #15 3 #20 Arg Phe Thr Leu Gly Thr Val Leu Gly Leu Al #a Thr Leu His Thr Tyr 325 # 330 # 335 Arg Gly Arg

Leu Ser Tyr Leu Pro Ala Thr Va #l Glu Pro Ala Ser Pro 340 # 345 # 350 Thr Pro Ala His Ser Leu Pro Arg Ala Lys Se #r Glu Leu Thr Leu Thr 355 # 360 # 365 Pro Asp Pro Ala Pro Pro Met Ala His Ser Pr #o Leu His Arg Ser Val 370 # 375 # 380 Ser Asp Leu Pro Leu Pro Leu Pro Gln Pro Al #a Leu Ala Ser Pro Gly 385 3 #90 3 #95 4 #00 Ser Pro Glu Pro Leu Pro Ile Leu Ser Leu As #n Gly Gly Gly Pro Glu 405 # 410 # 415 Leu Ala Gly Asp Trp Gly Gly Ala Gly Asp Al #a Pro Leu Ser Pro Asp 420 # 425 # 430 Pro Leu Leu Ser Ser Pro Pro Gly Ser Pro Ly #s Ala Ala Leu His Ser 435 # 440 # 445 Pro Val Ser Glu Gly Ala Pro Val Ile Pro Pr #o Ser Ser Gly Leu Pro 450 # 455 # 460 Leu Pro Thr Pro Asp Ala Arg Val Gly Ala Se #r Thr Cys Gly Pro Pro 465 4 #70 4 #75 4 #80 Asp His Leu Leu Pro Pro Leu Gly Thr Pro Le #u Pro Pro Asp Trp Val 485 # 490 # 495 Thr Leu Glu Gly Asp Phe Val Leu Met Leu Al #a Ile Ser Pro Ser His 500 # 505 # 510 Leu Gly Ala Asp Leu Val Ala Ala Pro His Al #a Arg Phe Asp Asp Gly 515 # 520 # 525 Leu Val His Leu Cys Trp Val Arg Ser Gly Il #e Ser Arg Ala Ala Leu 530 # 535 # 540 Leu Arg Leu Phe Leu Ala Met Glu Arg Gly Se #r His Phe Ser Leu Gly 545 5 #50 5 #55 5 #60 Cys Pro Gln Leu Gly Tyr Ala Ala Ala Arg Al #a Phe Arg Leu Glu Pro 565 # 570 # 575 Leu Thr Pro Arg Gly Val Leu Thr Val Asp Gl #y Glu Gln Val Glu Tyr 580 # 585 # 590 Gly Pro Leu Gln Ala Gln Met His Pro Gly Il #e Gly Thr Leu Leu Thr 595 # 600 # 605 Gly Pro Pro Gly Cys Pro Gly Arg Glu Pro 610 # 615 <210> SEQ ID NO 15 <211> LENGTH: 388 <212> TYPE: PRT <213> ORGANISM: Mus musculus <300> PUBLICATION INFORMATION: <302> TITLE: Molecular cloning and functional char #acterization of murine **sphingosine kinase** <303> JOURNAL: J. Biol. Chem. <304> VOLUME: 273 <305> ISSUE: 37 <306> PAGES: 23722-23728 <307> DATE: 1998-09-11 <308> DATABASE ACCESSION NUMBER: AAC61698 <309> DATABASE ENTRY DATE: 1998-09-26 <400> **SEQUENCE**: 15 Met Trp Trp Cys Cys Val Leu Phe Val Val Gl #u Cys Pro Arg Gly Leu 1 5 # 10 # 15 Leu Pro Arg Pro Cys Arg Val Leu Val Leu Le #u Asn Pro Gln Gly Gly 20 # 25 # 30 Lys Gly Lys Ala Leu Gln Leu Phe Gln Ser Ar #g Val Gln Pro Phe Leu 35

Claims Text - CLTX (11):

10. An isolated and purified DNA which encodes a peptide of a **sphingosine kinase** type 2 isoform, said DNA comprising a **sequence** selected from the group consisting of the **sequence** of Genbank Accession No. nkit325787 and the **sequence** of Genbank Accession No. bankit325752.

Claims Text - CLTX (51):

50. A diagnostic kit for detecting **sphingosine kinase** type 2 RNA/cDNA in a sample comprising primers or oligonucleotides specific for **sphingosine kinase** type 2 RNA or cDNA suitable for hybridization to **sphingosine kinase** type 2 RNA or cDNA and/or amplification of **sphingosine kinase** type 2 **sequences** and suitable ancillary reagents.

PGPUB-DOCUMENT-NUMBER: 20020042091

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20020042091 A1

TITLE: Methods and compositions for screening modulators of lipid kinases

PUBLICATION-DATE: April 11, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Normant, Emmanuel	Antony		FR	
Melendez, Alirio	Fresnes		FR	
Casamitjana, Olivier	Paris		FR	
Moreau, Francois	Issy Les Moulineaux		FR	

APPL-NO: 09/ 964860

DATE FILED: September 28, 2001

FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	DOC-ID	APPL-DATE
EP	00 402684.5	2000EP-00 402684.5	September 29, 2000

US-CL-CURRENT: 435/15

ABSTRACT:

The present invention relates to methods of screening compounds that modulate lipid kinases activity. The invention is more preferably based on the SPA technology to screen compounds that modulate the activity of lipid kinases, in particular membrane lipid kinases, more specifically sphingosine kinases. The invention also includes compositions, products, kits, etc for use in performing the above methods, as well as the compounds identified by said methods, and their uses.

---------- KWIC ---------

Detail Description Paragraph - DETX (32):

[0059] **Sphingosine kinase** may also be obtained from transfected cells containing a nucleic acid encoding said enzyme. In this regard, the nucleic acid **sequence** encoding a human **sphingosine kinase** has been described in Melendez et al. **GENE** 251, 19-24. The **sequence** may be transfected into cells, using various plasmids and/or vectors, containing various promoters, to produce the recombinant enzymes. The lysate of such cells (or other preparations derived therefrom) may be used in the screening assays of this invention.

Detail Description Paragraph - DETX (33):

[0060] In a preferred embodiment, the **Sphing sine Kinase** is obtained from procaryotic cells and preferably from insect cells such as Sf9 or Sf 21. The cells are transfected or infected by a vector comprising the nucleic acid **sequence** encoding the said enzyme . In this regard, baculovirus is a preferred vector and enzyme production is made according to "Baculovirus Expression Vectors" Davis R. O'REILLY, Lois K. MILLER, Verne A. LUCKOW incorporated herein by reference. However, any assay for protein production by baculovirus known by the skilled artisan is also usable in the contexte of the present invention.

PGPUB-DOCUMENT-NUMBER: 20020012984

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20020012984 A1

TITLE: Mammalian sphingosine - 1 - phosphate phosphatase

PUBLICATION-DATE: January 31, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Mandala, Suzanne M.	Scotch plains	NJ	US	
Thornton, Rosemary A.	Warren	NJ	US	

APPL-NO: 09/ 778171

DATE FILED: February 7, 2001

RELATED-US-APPL-DATA:

non-provisional-of-provisional 60180534 20000207 US

US-CL-CURRENT: 435/196, 435/325 , 435/6 , 435/7.92 , 536/23.2 , 800/8

ABSTRACT:

The present invention provides polynucleotides and polypeptides of a murine sphingosine-1-phosphate phosphatase, referred to herein as mSPP1. The polynucleotides and polypeptides are used to further provide expression vectors, host cells comprising the vectors, probes and primers, antibodies against the mSPP1 protein and polypeptides thereof, assays for the presence or expression of mSPP1 and assays for the identification of compounds that interact with mSPP1.

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit of U.S. Provisional Application No. 60/180,534 filed Feb. 7, 2000, the contents of which are incorporated herein by reference in their entirety.

---------- KWIC ---------

Summary of Invention Paragraph - BSTX (10):

[0007] Because of its dual function as a ligand and second messenger and its pivotal role in cell growth and survival, the synthesis and degradation of SPP is expected to be tightly regulated in a spatial-temporal manner. Until recently, however, little was known of the enzymes involved in SPP metabolism. A previous report described the purification of **sphingosine kinase** to apparent

homogeneity from rat kidney (Olivera, et al., (1998) J. Biol. Chem. 273, 12576-12583). Subsequently the first mammalian **sphingosine kinase** was cloned from rat and characterized (Kohama, et al., (1998) J. Biol. Chem. 273, 23722-23728). The kinase is described as belonging to a novel, highly conserved **gene** family (Kohama, et al., (1998) J. Biol. Chem. 273, 23722-23728 and Nagiec, et al., (1998) J. Biol. Chem. 273, 19437-19442). Enforced expression of the **sphingosine kinase** markedly enhanced the proliferation and survival of cells, substantiating the importance of intracellularly generated SPP in cell fate decisions (Olivera, et al., (1999) J. Cell Biol. 147, 545-548).

PGPUB-DOCUMENT-NUMBER: 20010041688

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20010041688 A1

TITLE: Methods and compositions for the regulation of vasoconstriction

PUBLICATION-DATE: November 15, 2001

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Waeber, Christian	Boston	MA	US	
Moskowitz, Michael A.	Belmont	MA	US	
Yoshimura, Shin-Ichi	Zurich	MA	CH	
Salomone, Salvatore	Somerville		US	

APPL-NO: 09/ 804987

DATE FILED: March 13, 2001

RELATED-US-APPL-DATA:

non-provisional-of-provisional 60188859 20000313 US

US-CL-CURRENT: 514/78, 424/85.1

ABSTRACT:

Methods and compositions for the treatment of conditions which would benefit from vasoconstriction or the inhibition of vasoconstriction via modulation of sphingosine kinase and sphingosine-1-phosphate phosphatase activity and EDG receptor signaling are provided. These conditions include migraine, stroke, subarachnoid hemorrhage and vasospasm. Also provided are screening methods for modulators of sphingosine kinase and sphingosine-1-phosphate phosphatase activity and EDG receptor signaling which are capable of regulating vasoconstriction.

RELATED APPLICATIONS

[0001] This application claims priority under Title 35 .sctn.119(e) of the U. S. Provisional Application No. 60/188,859, filed Mar. 13, 2000, and entitled "Methods and Compositions for the Regulation of Vasoconstriction", the entire contents of which are incorporated herein by reference.

---------- KWIC ---------

Detail Description Paragraph - DETX (13):

[0069] **Sphingosine kinase** is a protein which produces sphingosine-1-phosphate by the phosphorylation of sphingosine. **Sphing sine kinase** activators are compounds which up-regulate the activity, particularly the kinase activity, of **sphingosine kinase**. Kinase activity refers to the phosphorylation of substrates, and in the present case, kinase activity of **sphingosine kinase** results in the synthesis of sphingosine-1-phosphate. Agents that activate **sphingosine kinase** can function at a number of levels and in a number of different pathways including transcription and translation of **sphingosine kinase genes** and transcripts and post-translational modifications of **sphingosine kinase**. Other **sphingosine kinase** activators are **sphingosine kinase** agonists. **Sphingosine kinase** agonists bind **sphingosine kinase** and thereby enhance its kinase activity, ultimately upregulating the production of sphingosine-1-phosphate. Assays for transcriptional and/or translational activating factors have been described in the literature with respect to other **genes**. It is well within the skill of the ordinary artisan to adapt such techniques to the identification of activating factors specific for **sphingosine kinase**. Methods for measuring **sphingosine kinase** activity as well as methods for identifying **sphingosine kinase** agonists and antagonists are disclosed in PCT Patent Application No. PCT/AU98/00730 (WO 99/12533), the contents of which are incorporated herein by reference in their entirety. Examples of **sphingosine kinase** activators are tumor necrosis factor--alpha (i.e., TNF-.alpha.), epidermal growth factor (i.e., EGF), and platelet derived growth factor (i.e., PDGF).

Detail Description Paragraph - DETX (34):

[0090] One important category of agents are those which inhibit **sphingosine kinase** activity, and thereby interfere with the production of sphingosine-1-phosphate. These latter compounds are herein referred to as **sphingosine kinase** antagonists. Some known **sphingosine kinase** antagonists are agents which molecularly mimic the natural substrates of sphingosinse kinase. Such antagonists bind to **sphingosine kinase,** in some instances irreversibly, and thereby prevent the binding of natural substrates of **sphingosine kinase,** ultimately preventing the phosphorylation of these substrates. Examples of **sphingosine kinase** antagonists include methylsphingosine, N,N-dimethyl sphingosine, trimethylsphingosine, D,L-threo-dihydrosphingosine and high density lipoprotein. Other sphingosine derivatives that can be used as **sphingosine kinase** inhibitors are described in U.S. Pat. Nos. 5,583,160; 5,627,171; 5,466,716; 5,391,800; 5,137,919; 5,151,360; 5,248,824; 5,260,288; and 5,331,014. De Jonghe et al. disclose the use of short-chain sphingoid bases, including short chain sphinganine analogs and 3-fluoro-sphingosine analogs as inhibitors of **sphingosine kinase**. (De Jonghe et al., Bioorg Med Chem Lett 1999 9 (21):3175-3180) The invention embraces the use of such **sphingosine kinase** antagonists provided they are useful in the treatment of conditions benefiting by inhibition of vasoconstriction or increased vasodilation. Other **sphingosine kinase** antagonists may bind **sphingosine kinase** at sites other than the substrate binding site, provided they ultimately interfere with the catalytic activity of the kinase. A suitable **sphingosine kinase** antagonist may interfere with the catalytic activity of **sphingosine kinase** by interfering with or preventing the interaction with substrates or catalysts, or interfering or preventing the release of products, or by preventing the modification of the substrates by the enzyme. The cloning of

murine **sphingosine kinase** (GenBank Accession No. AF068748, AF068749) has been reported by Kohama et al., as have expression studies and activity studies aimed at measuring specific **sphingosine kinase** activity. (Kohama et al., J Biol Chem 1998 273 (37):23722-8) GenBank Accession Nos. NM.sub.--021972 and XM.sub.--012589 correspond to **sequences** of cloned human **sphing sine kinase**. Assays for any of the above agent classes have been described in the literature, and especially in PCT patent application Ser. No. PCT/AU98/00730 (WO 99/12533), the entire contents of which are incorporated herein by reference, which documents methods for measuring **sphingosine kinase** activity as well as methods for identifying **sphingosine kinase** agonists and antagonists.

Detail Description Paragraph - DETX (107):

[0163] As used herein, the term "antisense oligonucleotide" or "antisense" describes an oligonucleotide that is an oligoribonucleotide, oligodeoxyribonucleotide, modified oligoribonucleotide, or modified oligodeoxyribonucleotide which hybridizes under physiological conditions to DNA comprising a particular **gene** or to an mRNA transcript of that **gene** and, thereby, inhibits the transcription of that **gene** and/or the translation of that mRNA. The antisense molecules are designed so as to interfere with transcription or translation of a target **gene** upon hybridization with the target **gene** or transcript. Antisense oligonucleotides that selectively bind to either a nucleic acid molecule encoding an EDG receptor (preferably an EDG-3 receptor), a **sphingosine kinase** or a sphingosine-1-phosphate phosphatase are particularly preferred. Those skilled in the art will recognize that the exact length of the antisense oligonucleotide and its degree of complementarity with its target will depend upon the specific target selected, including the **sequence** of the target and the particular bases which comprise that **sequence**.

Detail Description Paragraph - DETX (108):

[0164] It is preferred that the antisense oligonucleotide be constructed and arranged so as to bind selectively with the target under physiological conditions, i.e., to hybridize substantially more to the target **sequence** than to any other **sequence** in the target cell under physiological conditions. Based upon the nucleotide **sequences** of nucleic acid molecules encoding EDG receptor, **sphingosine kinase** or sphingosine-1-phosphate phosphatase (e.g., GenBank Accession Nos. NM-005226, X83864, and AF184914 for EDG-3 receptor; AF068748, AF068749, NM.sub.--021972 and XM.sub.--012589 for **sphingosine kinase**; AF247177 for sphingosine-1-phosphate phosphatase) or upon allelic or homologous genomic and/or cDNA **sequences**, one of skill in the art can easily choose and synthesize any of a number of appropriate antisense molecules for use in accordance with the present invention. In order to be sufficiently selective and potent for inhibition, such antisense oligonucleotides should comprise at least about 10 and, more preferably, at least about 15 consecutive bases which are complementary to the target, although in certain cases modified oligonucleotides as short as 7 bases in length have been used successfully as antisense oligonucleotides. See Wagner et al., Nat. Med. 1(11):1116-1118, 1995. Most preferably, the antisense oligonucleotides comprise a complementary **sequence** of 20-30 bases. Although oligonucleotides may be chosen which are antisense to any region of the **gene** or mRNA transcripts, in preferred embodiments the antisense oligonucleotides correspond to N-terminal or 5'

upstream sites such as translation initiation, transcription initiation or promoter sites. In addition, 3'-untranslated regions may be targeted by antisense oligonucleotides. Targeting to mRNA splicing sites has also been used in the art but may be less preferred if alternative mRNA splicing occurs. In addition, the antisense is targeted, preferably, to sites in which mRNA secondary structure is not expected (see, e.g., Sainio et al., Cell Mol. Neurobiol. 14(5):439-457, 1994) and at which proteins are not expected to bind.

Detail Description Paragraph - DETX (114):

[0170] The invention also embraces the use of **gene** therapy to increase the expression of EDG receptors (preferably EDG-3 receptor), **sphingosine kinase** and/or sphingosine-1-phosphate phosphatase. The procedure for performing ex vivo **gene** therapy is outlined in U.S. Pat. No. 5,399,346 and in exhibits submitted in the file history of that patent, all of which are publicly available documents. In general, it involves introduction in vitro of a functional copy of a **gene** into a cell(s) of a subject which, in some instances, contains a defective copy of the **gene**, and returning the genetically engineered cell(s) to the subject. The functional copy of the **gene** is under operable control of regulatory elements which permit expression of the **gene** in the genetically engineered cell(s). Numerous transfection and transduction techniques as well as appropriate expression vectors are well known to those of ordinary skill in the art, some of which are described in PCT application WO 95/00654. In vivo **gene** therapy using vectors such as adenovirus also is contemplated according to the invention.

Detail Description Paragraph - DETX (143):

[0188] The sphingolipid sphingosine-1-phosphate acts as a messenger through stimulation of G-protein coupled EDG (Endothelial Differentiation **Gene**) receptors. These experiments indicate that sphingosine-1-phosphate causes robust constriction of isolated cerebral, but not peripheral arteries. This selectivity could be explained by the lower expression of sphingosine-1-phosphate phosphatase in basilar artery, as assessed by RT-PCR. Remarkably, sphingosine fully prevents the vasoconstrictive response to sphingosine-1-phosphate. Therefore sphingosine, the precursor of sphingosine-1-phosphate, antagonizes the action of its phosphorylated derivative, whereas **sphingosine kinase** acts as a converting enzyme modulating vascular contractility. Basilar artery constriction by sphingosine-1-phosphate is specifically reduced by edg-3 antisense-treatment, showing that EDG-3 receptors mediate this response. Consistent with sphingosine-1-phosphate mediated constriction and release from platelets during clotting, a significant reduction in cerebral infarct size in an embolic stroke model was observed when rats were pre-treated with a **sphingosine kinase** inhibitor. Hence, sphingosine-1-phosphate/sphingosine/EDG-3 system provides a useful therapeutic target for the treatment of thrombo-embolic insults in brain. Moreover, EDG-3 receptor antagonists, **sphingosine kinase** inhibitors and drugs which promote S1P phosphatase activity are novel therapeutic strategies for the treatment of stroke and other related cerebrovascular diseases.

apoptosis: The AV3SK vector and its enzymatic product, sphingosine 1-phosphate (S-1-P) were both evaluated for possible inhibition of apoptosis in human cardiac myocytes induced by ceramide (n=3). heat shock (n=3), ischemia/reoxygenation (n=2). For vector-mediated studies, experiments were performed 3 days after vector treatment. The data, shown in FIG. 6, indicate that S-1-P is a potent inhibitor of human cardiac myocyte cell death. Av3SK transduced cells are also almost completely resistant to heat shock and ischemia/reoxygenation-induced apoptosis (FIG. 6). However, Av3SK vectors can only partially inhibit ceramide-induced apoptosis. This data in cardiac myocytes supports a cardioprotective role for **sphingosine kinase** and S1P. The use of a **gene** therapy vector to express **sphingosine kinase** represents a treatment modality for the long-term protection of cardiac myocytes from injury and protect against congestive heart failure.

US-PAT-NO: 6534323

DOCUMENT-IDENTIFIER: US 6534323 B1

TITLE: Compositions and methods for early detection of heart disease

DATE-ISSUED: March 18, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Sabbadini; Roger	Lakeside	CA	N/A	N/A

APPL-NO: 09/ 489466

DATE FILED: January 21, 2000

PARENT-CASE:

This application is related and claims priority to U.S. Provisional Application No. 60/049,274, filed on Jun. 10, 1997; and a Div. U.S. Application No. 09/084,069, filed on May 22, 1998, which issued as U.S. Pat. No. 6,210,976 B1 on Apr. 3, 2001.

US-CL-CURRENT: 436/518, 435/7.1 , 435/7.92 , 435/810 , 435/967 , 435/975 , 436/161 , 436/162 , 436/173 , 436/536 , 436/541 , 436/63 , 436/71 , 436/808 , 530/387.1 , 530/388.1 , 530/388.25 , 530/389.1 , 530/389.3 , 530/391.1 , 530/412 , 530/413 , 530/417

ABSTRACT:

The invention relates to methods, compositions, kits, and devices for detecting cardiac ischemia, hypoxia, or other causes of heart failure in a mammal by obtaining a test sample from a mammal, measuring a level of a non-polypeptidic cardiac marker in the test sample, and determining if the level of the cardiac marker measured in said test sample correlates with cardiac ischemia or hypoxia or another form of heart failure.

28 Claims, 1 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 1

---------- KWIC ---------

Detailed Description Text - DETX (116):

It has been shown that sphingosine inhibits PKC by preventing DAG binding to the enzyme (Faucher et al., J. Biol. Chem. 263:5319-5327, 1988). Thus, sphingosine may bind directly to PKC via the DAG binding site. The **sequence** for PKC.alpha. and its consensus DAG binding site is known (Hurley et al., Protein Science (6):477-80, 1997). Since SPH can also bind to putative sites on **sphingosine kinase** and other proteins with which is specifically interacts, it quite likely that several proteins have specific SPH binding sites. Accordingly, the putative sphingolipid binding site can be cloned using standard techniques, after the screening of phage display libraries (see above) for colonies, which express the sphingolipid recognition site. Expression cloning of the cDNA of this protein would produce a reagent that could be used in a standard ELISA to detect sphingolipid changes in a blood sample.

US-PAT-NO: 6534322

DOCUMENT-IDENTIFIER: US 6534322 B1

TITLE: Kits for early detection of heart disease

DATE-ISSUED: March 18, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Sabbadini; Roger A.	Lakeside	CA	N/A	N/A

APPL-NO: 09/ 489158

DATE FILED: January 21, 2000

PARENT-CASE:

RELATED APPLICATIONS

This application is a divisional of U.S. patent application Ser. No. 09/084,069, filed May 22, 1998, by Sabbadini et al., and entitled "METHODS FOR EARLY DETECTION OF HEART DISEASE" now U.S. Pat. No. 6,210,976, which in turn claims priority to the U.S. Provisional Patent Application Serial No. 60/049,274, filed Jun. 10, 1997, by Sabbadini, et al., and entitled "DIAGNOSIS OF HEART DISEASES USING SPHINGOLIPIDS," both of which are hereby incorporated by reference herein in their entirety, including any drawings.

US-CL-CURRENT: 436/518, 435/7.1 , 435/7.92 , 435/810 , 435/967 , 435/975 , 436/161 , 436/162 , 436/173 , 436/536 , 436/541 , 436/63 , 436/71 , 436/808 , 530/412 , 530/413 , 530/417

ABSTRACT:

The invention relates to methods, compositions, kits, and devices for detecting cardiac ischemia, hypoxia, or other causes of heart failure in a mammal by obtaining a test sample from a mammal, measuring a level of a non-polypeptidic cardiac marker in the test sample, and determining if the level of the cardiac marker measured in said test sample correlates with cardiac ischemia or hypoxia or another form of heart failure.

16 Claims, 1 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 1

---------- KWIC ---------

Detailed Description Text - DETX (117):

It has been shown that sphingosine inhibits PKC by preventing DAG binding to the enzyme (Faucher et al., J. Biol. Chem. 263:5319-5327, 1988). Thus, sphingosine may bind directly to PKC via the DAG binding site. The **sequence** for PKC.alpha. and its consensus DAG binding site is known (Hurley et al., Protein Science (6):477-80, 1997). Since SPH can also bind to putative sites on **sphingosine kinase** and other proteins with which is specifically interacts, it quite likely that several proteins have specific SPH binding sites. Accordingly, the putative sphingplipid binding site can be cloned using standard techniques, after the screening of phage display libraries (see above) for colonies, which express the sphingolipid recognition site. Expression cloning of the cDNA of this protein would produce a reagent that could be used in a standard ELISA to detect sphingolipid changes in a blood sample.

US-PAT-NO: 6482609

DOCUMENT-IDENTIFIER: US 6482609 B1

TITLE: Isolated human EDG-4 receptor and polynucletide encoding said receptor

DATE-ISSUED: November 19, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Munroe; Donald G.	Waterdown	N/A	N/A	CA
Kamboj; Rajender	Mississauga	N/A	N/A	CA
Peters; Diana	Toronto	N/A	N/A	CA
Kooshesh; Fatemch	Etobicoke	N/A	N/A	CA
Vyas; Tejal B.	Mississauga	N/A	N/A	CA
Gupta; Ashwani K.	Mississauga	N/A	N/A	CA

APPL-NO: 09/ 582200

DATE FILED: July 28, 2000

PARENT-CASE:

This application is a 371 of WO 99/35259 filed on Dec. 30, 1998, which claims priority from provisioned application No. 60/070185, filed Dec. 30, 1997 which claims priority from provisioned application No. 60/080,610 filed Apr. 3, 1998 which claims priority from provisional application No. 60/109,885 filed Nov. 25, 1998.

PCT-DATA:
APPL-NO: PCT/CA98/01195
DATE-FILED: December 30, 1998
PUB-NO: WO99/35259
PUB-DATE: Jul 15, 1999
371-DATE:
102(E)-DATE:

US-CL-CURRENT: 435/69.1, 435/252.2 , 435/320.1 , 435/325 , 435/471 , 435/71.1 , 530/350 , 536/23.4 , 536/23.5

ABSTRACT:

A lysolipid receptor, a human EDG-4 receptor, a method of identifying lysolipid receptors involved in inflammatory response and the lysolipid receptors so identified, and a method of identifying ligands which interact with such lysolipid receptors.

16 Claims, 36 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 36

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Detailed Description Text - DETX (217):

Like p75.sup.NGFR, several other death receptors have been shown to induce apoptosis and/or NF-.kappa.B activation, depending on the cell type and costimulus applied. The involvement of sphingomyelinase, ceramide/sphingosine and **sphingosine kinase** in the signaling cascade has also been shown repeatedly with TNFR, Fas/CD95 and other family members. Another parallel with the NGF system is the observation that some cell types that express a given death receptor survive their ligands while other do not. Again, protein kinase C is implicated in survival pathways. There is even direct evidence that S1P plays a similar role in survival for Fas/CD95 and in inflammatory **gene** expression for TNFR. Therefore, one can predict a widespread role for inflammatory lysosphingolipid/edg receptors in modulating the apoptotic/inflammatory potential of death receptor ligands. If true, these GPCRs may play a fundamental role in cell survival, differentiation, and inflammation. Therefore, methods for isolating such receptors, and for identifying ligands that modulate these activities constitute aspects of the invention described herein.

Detailed Description Text - DETX (240):

References An, S, Bleu, T, Huang, W, Hallmark, O G, Coughlin, S R and Goetzl, E J. 1997. Identification of cDNAs encoding two G protein-coupled receptors for lysosphingolipids. FEBS Lett 417: 279-282. Carter, B D, Kaltschmidt, C, Kaltschmidt, B, Offenhauser, N, Bohm-Matthaei, R, Baeuerle, P and Barde, Y-A. 1996. Selective activation of NF-.kappa.B by nerve growth factor through the neurotrophin receptor p75. Science 272: 542-545. Cuvillier, O, Pirianov, G, Kleuser, B, Vanek, P G, Coso, O A, Gutkind, J S and Spiegel, S. 1996. Suppression of programmed cell death by sphingosine-1-phosphate. Nature 381: 800-803. Cuvillier, O, Rosenthal, D S, Smulson, M E and Spiegel, S. 1998. Sphingosine 1-phosphate inhibits activation of caspases that cleave poly(ADP-ribose) polymerase and lamins during Fas- and ceramide-mediated apoptosis in Jurkat T lymphocytes. J Biol Chem 273: 2910-2916. Edsall, L C, Pirianov, G G and Spiegel, S. 1997. Involvement of sphingosine 1-phosphate in nerve growth factor-mediated neuronal survival and differentiation. J Neurosci 17: 6952-6960. Hla, T and Maciag, T. 1990. An abundant transcript induced in differentiating human endothelial cells encodes a polypeptide with structural similarities to C-protein-coupled receptors. J Biol Chem 265: 9308-9313. Lee, M-J, Van Brocklyn, J R, Thangada, S, Liu, C H, Hand, A R, Menzeleev, R, Spiegel, S and Hla T. Sphingosine-1-phosphate as a ligand for the G protein-coupled receptor EDG-1. Science 279: 1552-1555. MacLennan, A J. 1996. Molecular cloning and expression of G-protein coupled receptors. U.S. Pat. No. 5,585,476. Issued Dec. 17, 1996. MacLennan, A J, Browe, C S, Gaskin, A A, Lado, D C and Shaw, G. 1994. Cloning and characterization of a putative G-protein coupled receptor potentially involved in development. Mol Cell Neurosci 5: 201-209. MacLennan, A J, Marks, L,

Gaskin, A A and Lee, N. 1997. Embryonic expression pattern of H218, a G-protein coupled receptor homolog, suggests roles in early mammalian nervous system development. Neuroscience 79: 217-224. Okazaki, H, Ishizaka, N, Sakurai, T, Kurokawa, K, Goto, K, Kumada, M and Takuwa, Y. 1993. Molecular cloning of a novel putative G protein-coupled receptor expressed in the cardiovascular system. Biocehm Biophys Res Commun 190: 1104-1109. Rius, R A, Edsall, L C and Spiegel, S. 1997. Activation of **sphingosine kinase** in pheoclromocytoma PC12 neuronal cells in response to trophic factors. FEBS Lett 417: 173-176. Shatrov, V A, Lehmann, V and Chouaib, S. 1997. Sphingosine-1-phosphate mobilizes intracellular calcium and activates transcription factor NF-.kappa.B in U937 cells. Biochem Biophys Res Commun 234: 121-124. Spiegel, S. 1998. Use of sphingosine-1-phosphate to suppress programmed cell death. U.S. Pat. No. 5,712,262. Issued Jan. 27, 1998. Taglialatela, G, Robinson, R and Perez-Polo, J R. 1997. Inhibition of nuclear factor kappa B (NF.kappa.B) activity induces nerve growth factor-resistant apoptosis in PC12 cells. J Neurosci Res 47: 155-162 Yamaguchi, F, Tokuda, M, Hatase, O and Brenner, S. 1996. Molecular cloning of the novel human G protein-coupled receptor (GPCR) **gene** mapped on chromosome 9. Biochem Biophys Res Commun 227: 608-614. Zondag, G C M, Postma, F R, van Etten, I, Verlaan, I and Moolenaar, W H. 1998. Biochemi J 330

US-PAT-NO: 6210976

DOCUMENT-IDENTIFIER: US 6210976 B1

TITLE: Methods for early detection of heart disease

DATE-ISSUED: April 3, 2001

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Sabbadini; Roger A.	Lakeside	CA	N/A	N/A

APPL-NO: 09/ 084069

DATE FILED: May 22, 1998

PARENT-CASE:

RELATED APPLICATIONS

This application is related and claims priority to U.S. Provisional Application No. 60/049,274, by Sabbadini, entitled "METHOD FOR EARLY DETECTION OF MYOCARDIAL ISCHEMIA," and filed on Jun. 10, 1997.

US-CL-CURRENT: 436/518, 435/7.1 , 435/7.92 , 435/7.93 , 435/7.94 , 436/71 , 436/811 , 436/815

ABSTRACT:

The invention relates to methods, compositions, kits, and devices for detecting cardiac ischemia, hypoxia, or other causes of heart failure in a mammal by obtaining a test sample from a mammal, measuring a level of a non-polypeptidic cardiac marker in the test sample, and determining if the level of the cardiac marker measured in said test sample correlates with cardiac ischemia or hypoxia or another form of heart failure.

40 Claims, 1 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 1

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Detailed Description Text - DETX (117):

It has been shown that sphingosine inhibits PKC by preventing DAG binding to the enzyme (Faucher et al., J. Biol. Chem. 263:5319-5327, 1988). Thus, sphingosine may bind directly to PKC via the DAG binding site. The **sequence**

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